Trends in Microbiology

CellPress REVIEWS

Review

The Gut Microbiota in the First Decade of Life

Muriel Derrien,[1,*] Anne-Sophie Alvarez,[1] and Willem M. de Vos [2,3]

Appreciation of the importance of the gut microbiome is growing, and it is becoming increasingly relevant to identify preventive or therapeutic solutions targeting it. The composition and function of the gut microbiota are relatively well described for infants (less than 3 years) and adults, but have been largely overlooked in pre-school (3–6 years) and primary school-age (6–12 years) children, as well as teenagers (12–18 years). Early reports suggested that the infant microbiota would attain an adult-like structure at the age of 3 years, but recent studies have suggested that microbiota development may take longer. This development time is of key importance because there is evidence to suggest that deviations in this development may have consequences in later life. In this review, we provide an overview of current knowledge concerning the gut microbiota, its evolution, variation, and response to dietary challenges during the first decade of life with a focus on healthy pre-school and primary school-age children (up to 12 years) from various populations around the globe. This knowledge should facilitate the identification of diet-based approaches targeting individuals of this age group, to promote the development of a healthy microbiota in later life.

Highlights

The gut microbiota of healthy children displays functional and taxonomic differences with respect to those of adults, suggesting that the gut microbiome may develop more slowly than previously thought.

Bifidobacterium spp. are more abundant in the gut microbiota of children than in that of adults, and may gradually decrease until adulthood.

The microbiota may develop more slowly in some children than in others, who may present an intermediate microbiota state.

Childhood may provide additional opportunities for microbiota-based interventions to promote health or prevent microbiota deviation.

The gut microbiota of children may be more malleable to environmental factors than that of adults.

Differences in lifestyle, and westernization in particular, strongly influence the composition of gut microbial populations in children, as already reported for adults.

The Role of the Microbiota in Lifelong Health

Most microorganisms live in a complex community, called microbiota, which consists of bacteria, archaea, and fungi, but also includes their viruses and phages. The microbiota of the human intestinal tract is no exception and forms a dense ecosystem dominated by bacteria [1]. Considerable attention has recently been focused on the gut microbiota, which has been implicated in the regulation of multiple host pathways [2,3]. From birth onwards, the gut microbiota coevolves with the host and the host's metabolic and neurological programming; the development of this microbial community is thus of crucial importance for health in later life [4,5]. The development of the gut microbiota is regulated by a complex interplay between the host and environmental factors, including diet and lifestyle [6]. Hence, the dynamics of the gut microbiota from birth until old age can shed light on the variation of this community within the host and possible associations with disease risks. In the past decade, studies on the development of the infant–gut microbiota symbiosis during the first 3 years of life have greatly advanced, particularly through the use of large and longitudinal cohorts in conjunction with deep metagenomic or phylogenetic analyses, facilitating high-resolution investigations of the composition, function, and origin of the microbiota [7–9].

The infant–gut microbiota symbiosis is established from birth and is shaped during the first few years of life [10]. During this period, infants grow rapidly, displaying large increases in height, weight, and head circumference, and their metabolic organs, immune system, digestive system, and neurologic and cognitive abilities go through major changes as they develop and mature. This is also a key period for the establishment of the gut microbiota and subsequent good health. The gut microbiota influences the maturation of the immune system, nutrient absorption, and metabolism, and prevents pathogen colonization [11]. Several studies and reviews have highlighted the crucial nature of the development of this symbiosis in early life for infant health and its lifelong consequences [12,13]. Changes in the composition of the gut microbiota

[1]Danone Nutricia Research, RD, 128 Avenue de la Vauve, 91120 Palaiseau, France
[2]Laboratory of Microbiology, Wageningen University and Research, Wageningen, The Netherlands
[3]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland

*Correspondence: muriel.derrien@danone.com (M. Derrien).

have been associated with short- and long-term health disorders, such as being overweight, obesity, atopy manifestations, asthma, metabolic syndromes, and chronic inflammatory diseases [14–16]. Thus, early life provides a unique window of opportunity for modulating the gut microbiota to promote long-term health.

The gut microbiota of adults in different parts of the world has been studied in much more detail than that of children. It has been suggested that the microbiota is relatively stable and resilient in adults, in the absence of extreme external stressors (e.g., dietary changes or antibiotic treatment) [17,18]. This considerable resilience enables it to return to its original state when a challenge ceases [19,20]. The recent emergence of large population-based cohorts, including large panels of multiple types of host and environmental metadata, collected from thousands of subjects, has greatly increased our insight into microbial ecology and accelerated the identification of external factors associated with variation in microbiota [21–23]. Recent discoveries relating to the evolution of the gut microbiota in elderly individuals have provided additional insight into the multiple trajectories of the microbiota during the course of a human lifespan [24]. These studies have paved the way for an understanding of the variation of the microbiota in healthy individuals and the design of microbiota-based interventions throughout an individual's lifetime. By contrast, we still know little about the variation of the microbiota in children over the age of 3 years (Figure 1) and its response to challenges; this is probably because such investigations have been constrained by ethical and practical considerations, such as difficulties in obtaining fecal samples from individuals in these age groups, particularly around puberty [25]. Nevertheless, interest in this specific period of life has increased, with cross-sectional and longitudinal studies in different populations, and analyses of associations with environmental factors, including dietary habits.

In this review, we provide an overview of current knowledge concerning the variation and evolution of the gut microbiota during the first decade of life, with a focus in pre-school children (3–6 years) and primary school-age children (6–12 years), focusing on those in good health. We also discuss the environmental factors, including diet in particular, associated with variation in the microbiota, and the plasticity of the microbiota in response to dietary interventions. This knowledge may facilitate the design of microbiota-based interventions to promote health or prevent diseases in older age.

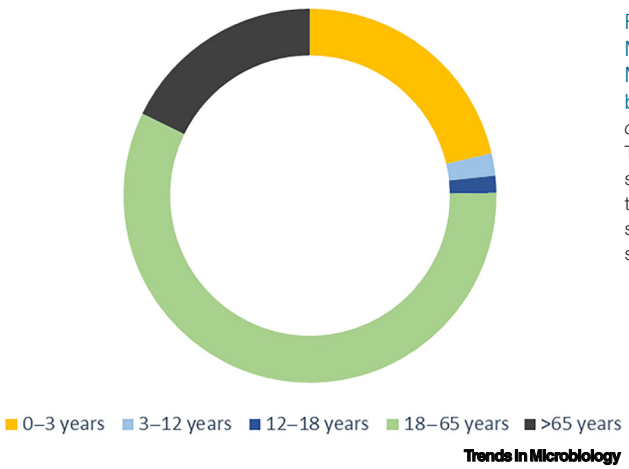

Figure 1. Proportion of Gut Microbiota Samples Analyzed by Metagenomic Shotgun Sequencing, by Age Category from the *curatedMetagenomicData* package [99]. The data are limited to shotgun sequencing, but they show disparities in the prevalence of samples, stratified by subject age category, compiled within a single database.

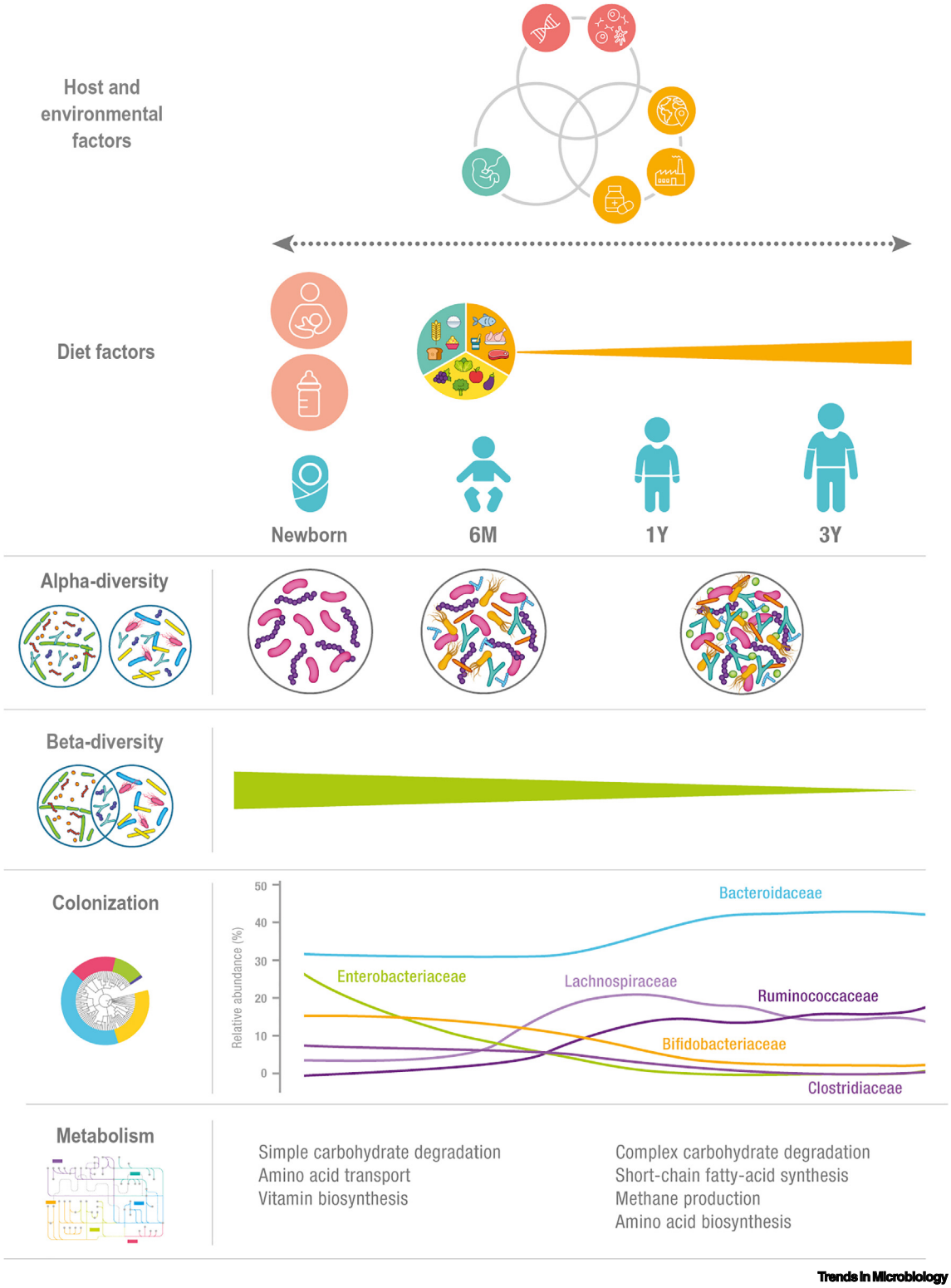

Figure 2. Summary of Microbiota Development within the First 3 Years of Life. Bacterial alpha-diversity and functional complexity increase with age, while interindividual variations (beta-diversity) decrease. Colonization pattern is based on [100].

Establishment of the Infant Gut–Microbiota Symbiosis: Diet as a Modulator of Gut Microbiota Development

The development and maturation of the gut microbiota are highly dynamic processes and they are influenced by various perinatal conditions, including external factors (e.g., mode of delivery, type of feeding, antibiotic use, lifestyle, and geographic factors) [26] and host factors [27,28]. Many studies have monitored the dynamics of the gut microbiota during the first 3 years of life in health and disease, and have revealed common patterns of development across countries, as recently reviewed in detail elsewhere [15,29] (Figure 2).

Here, we discuss briefly the shaping of the development of the microbiota during the first few years of life by several factors, such as diet in particular, which exerts a selection effect favoring the gut microbes best adapted to the dynamic conditions in the intestine. Several studies have shown that maternal milk protects against infection in infants, due to the presence of immune effectors, such as immunoglobulin A (IgA) [30]. In addition, this natural mode of feeding contributes to the maturation of the infant's immune system and modulates the development of its gut microbiota. Indeed, it has been well established that the gut microbiota differs between formula-fed and breastfed infants. The gut microbiota of breastfed infants is less diverse, but it includes higher levels of *Bifidobacterium* species, including *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* in particular, these species being the most abundant and able to thrive on human milk oligosaccharides (HMOs) [7,8,31,32]. HMO composition is affected by genetic factors, such as secretor genotype, providing a rationale for the effect of the mother's genome on the gut microbiota of her infant [33]. Strains of *B. breve*, *B. longum*, and *B. bifidum* were recently reported to have different sugar-use profiles, suggesting that differences in nutrient availability between infants can promote colonization by specific *Bifidobacterium* species [34]. The specialization of *B. longum* susbp *infantis* in HMO metabolism is an example of adaptation to a specific ecological niche. This bacterium contains a large repertoire of genes for import and intracellular metabolism. Four glycosyl glycosidase genes (sialidase, fucosidase, *N*-acetyl-β-hexosaminidase, β-galactosidase) and transport-related genes (solute-binding proteins and ABC transporters) are localized in an HMO metabolism cluster [35]. Interestingly, the prevalence of *B. longum* subsp *infantis* varies across populations, and this subspecies is present in about 10% of Finnish, 20% of Estonian, and 23% of Russian infants [34]. The supplementation of formula milk with galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), at a 9:1 ratio, appears to mimic, to some extent, the effect of the HMO of formula milk on the gut microbiota, including *Bifidobacterium* stimulation [36]. During breastfeeding, the gut microbiota is equipped for the metabolism of lactate and plant-derived glycans, including starch, indicating that the microbial communities are metabolically ready for the introduction and metabolism of simple plant-derived foods [37]. The cessation of breastfeeding, rather than the introduction of solid food, has been reported to be a major event influencing the microbiota [7,8]. The introduction of solid food is associated with a higher bacterial load and diversity [31,37], higher total short-chain fatty acid levels, and a dominance of Bacteroides/Firmicutes, which are better able to break down complex carbohydrates [31]. Close monitoring of microbiota development in 98 Swedish infant–mother pairs over a period of 1 year revealed an increase in the capacity to produce amino acids and vitamins at the age of 4 months [7]. At the age of 12 months, these infants showed an enrichment in the expression of genes involved in the degradation of complex sugars and starch, which was associated with a higher abundance of *Bacteroides thetaiotaomicron*, a species known to harbor a large repertoire of glycan-degrading enzymes and to be capable of degrading HMOs [38]. Additional metabolic pathways relating to vitamin biosynthesis and xenobiotic metabolism are also expressed following the introduction of solid food, reflecting the diversity of substrates in the adult diet [37]. Interestingly, the gut microbiome of infants (up to 3 years of age) from different populations has been shown to be enriched in genes involved in the *de novo*

biosynthesis of folate (vitamin B9), whereas those of adults contain a significantly higher proportion of genes encoding proteins responsible for the metabolism of dietary folate. Genes encoding cobalamin (vitamin B12) were found to become more abundant with age [39].

Collectively, these studies indicate a gradual specialization of the microbiota to deal with the substrates available in the gut. *Bifidobacterium* spp. are chiefly responsible for HMO metabolism, but diverse bacteria act together to break down proteins, plant-derived complex carbohydrates, and resistant starch. Many studies have highlighted a lower bacterial diversity, a lower functional complexity, and a higher degree of interpersonal variation in gut bacterial diversity between infants than between adults [7,31,39,40]. Dedicated metrics ('relative microbiota maturity' and 'microbiota-for-age Z score') have been developed, based on a microbial signature of the state of maturation of an individual's microbiota relative to that of a reference group of similar age. These metrics were first applied to infants with severe acute malnutrition, and the results obtained suggested that these infants had a less-mature microbiota than age-matched healthy infants [41]. This approach was subsequently used in other studies. Microbiota age was found to be greater in formula-fed infants than in those breastfed [7,8], and in infants delivered by cesarean section than in those delivered by the vaginal route [7]. Other life events, including maturation of the immune response and the gut epithelium [42], may account for differences in colonization patterns between infant gut microbiota, and are not discussed in the current review.

Bridging the Gap between Early Life and Adulthood: The Case of Healthy Pre-school and Primary School-age Children

Several studies have reported an apparent stabilization of the gut microbiota in an adult-like configuration within the first 3 years of life [7,37,39]. One of the largest and most comprehensive analyses of gut microbiota development to date included 903 infants from four countries (Germany, Finland, Sweden, USA) followed for the first 3 years of life by monthly stool sampling [8]. This study revealed that the gut microbiota evolves in three distinct phases based on the dynamics of the most abundant phyla (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia) and changes in alpha-diversity: a developmental phase (months 3–14) in which the phyla detected and alpha-diversity gradually change, a transitional phase (months 15–30) in which only Bacteroidetes and Proteobacteria continue to develop and alpha-diversity continues to change, and a stable phase (≥31 months) in which the phyla present and alpha-diversity remain unchanged. *Bifidobacterium* spp. dominate during the developmental phase, whereas the stable phase is characterized by a higher bacterial diversity and a predominance of the Firmicutes. These findings suggest that most of the development of gut microbiota composition and function occurs within the sampling period of 3 years. However, recent longitudinal studies with sampling over longer time periods have suggested that complete maturation of the gut microbiota might take longer, particularly for some of its members. In this section, we focus on studies that extended the monitoring of the gut microbiota beyond the age of 3 years and compared subjects in early and late childhood with adults [43–46] (Figure 3).

In a study addressing the composition of the gut microbiota of children between the ages of 1 and 4 years and adults from the USA, it was found that members of the Actinobacteria, Bacilli, *Clostridium* cluster IV (Ruminococcaceae), and Bacteroidetes were more prevalent in children than in adults. In contrast, members of *Clostridium* cluster XIVa (*Butyrivibrio crossotus* and related bacteria) were more abundant in adults than in children [44]. These findings suggest that some members of the microbiota may already be established in young children, whereas others are continuing to evolve. In this study, the older children studied (3–4 years of age) still had a lower microbial diversity, with a higher relative abundance of *Bifidobacterium,* than adults. In another study, monitoring of the gut microbiota of 28 children was extended to the age of 5 years [43].

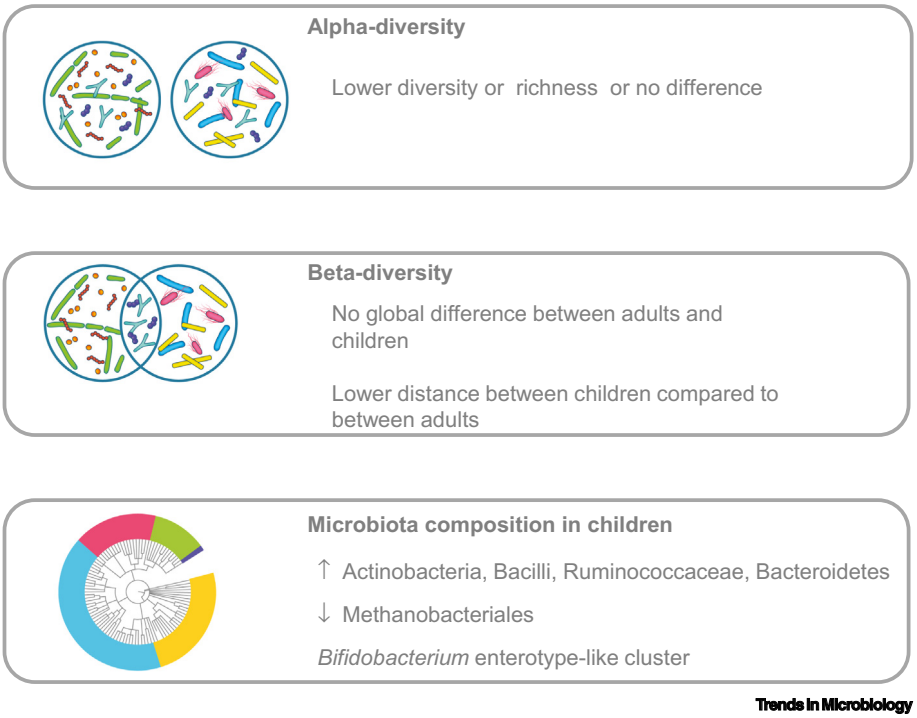

Figure 3. Summary of Converging Findings from Major Clinical Studies Comparing the Gut Microbiota of Pre-school and School-age Children with That of Adults.

Interestingly, microbial diversity at 5 years of age was still significantly lower than that in adults. These findings are consistent with the previous one [44], with the same taxa, including Actinobacteria, Bacilli, and *Clostridium* cluster IV, retaining abundances similar to those in infants, and others, such as *Clostridium* cluster XIVa (Lachnospiraceae), adopting a distribution more like that in adults [43]. In a recent study, Hollister *et al.* surveyed the gut microbiome from older children (7–12 years of age) combining both taxonomic and functional analyses. *Bifidobacterium* and *Faecalibacterium* spp. were found to be significantly more abundant in children than in adults, whereas adults display an enrichment in *Bacteroides* (i.e., *Bacteroides vulgatus* and *Bacteroides xylanisolvens*) [45]. The relative abundance of genes involved in functions such as vitamin synthesis (B9 and B12), amino-acid degradation, oxidative phosphorylation, and mucosal inflammation differ between children and adults. Overall, these findings indicate that the gut microbiota of healthy children of around 3 years of age continues to display functional and taxonomic differences as compared with those of adults, suggesting that the gut microbiome may develop more slowly than previously thought.

The follow-up of prospective birth cohorts provides an opportunity for the comprehensive analysis of the gut microbiota in children as they age. The KOALA Birth Cohort Study has generated an extensive collection of both host and environmental metadata, including data for the gut microbiota from a subset of subjects [47]. The largest ever comprehensive cross-sectional analysis of gut microbiota from 281 school-age children (6–9 years) from this birth cohort was recently compared with data from adults [46]. This study showed that the overall structure of the microbiota, as assessed by beta-diversity metrics, was similar to that in adults. Overall, the gut microbiota of school-age children was found to be enriched in taxa from the Bacteroidetes and Actinobacteria (*Bifidobacterium*) (Figure 4) and to have a functional composition similar to that in healthy adults. These findings contrast with previous reports of a significantly lower abundance

of Bacteroidetes in healthy children aged 7–12 years than in healthy adults, and in children aged 1–4 years [44,45]. Intra-group similarity is higher in children than in adults, suggesting that the microbiota of children is more similar to each other than to those of adults, as previously reported [45].

The intestinal microbiota of adults has been reported to stratify into clusters, referred to as enterotypes and driven by one of the following genera: *Bacteroides*, *Prevotella*, and *Ruminococcus* [48,49]. In a recent study of the microbiota of European infants, *Prevotella* and *Bacteroides* enterotypes were predicted to develop between 9 and 36 months [50]. A study of Asian school-age children suggested the presence of *Bifidobacterium/Bacteroides* and *Prevotella* enterotype-like clusters, the prevalence of which depended on geographic regions [51]. Recently, three enterotype-like clusters were detected in the microbiota of school-aged Dutch children (*Bacteroides, Prevotella*, and *Bifidobacterium*). Children harboring microbiota enriched in the *Bifidobacterium* enterotype-like cluster (22% of the children) were found to have a lower microbiota richness and diversity [46]. Functionally, they displayed an enrichment in pathways related to the metabolism of simple sugars, including glycolysis and the pentose-phosphate pathway, whereas pathways related to the utilization of complex carbohydrates, such as pectin, uronic acids, and glycosaminoglycan, were depleted. This suggests that children with the *Bifidobacterium* enterotype-like cluster have a less mature microbiota than children with the *Prevotella* and *Bacteroides* enterotypes, but it is not known which factors are associated with the emergence of adult-like enterotypes. This study also showed that early-life and pre-school events, including the duration of breastfeeding in particular, were associated with the microbiota composition of school-age children [46].

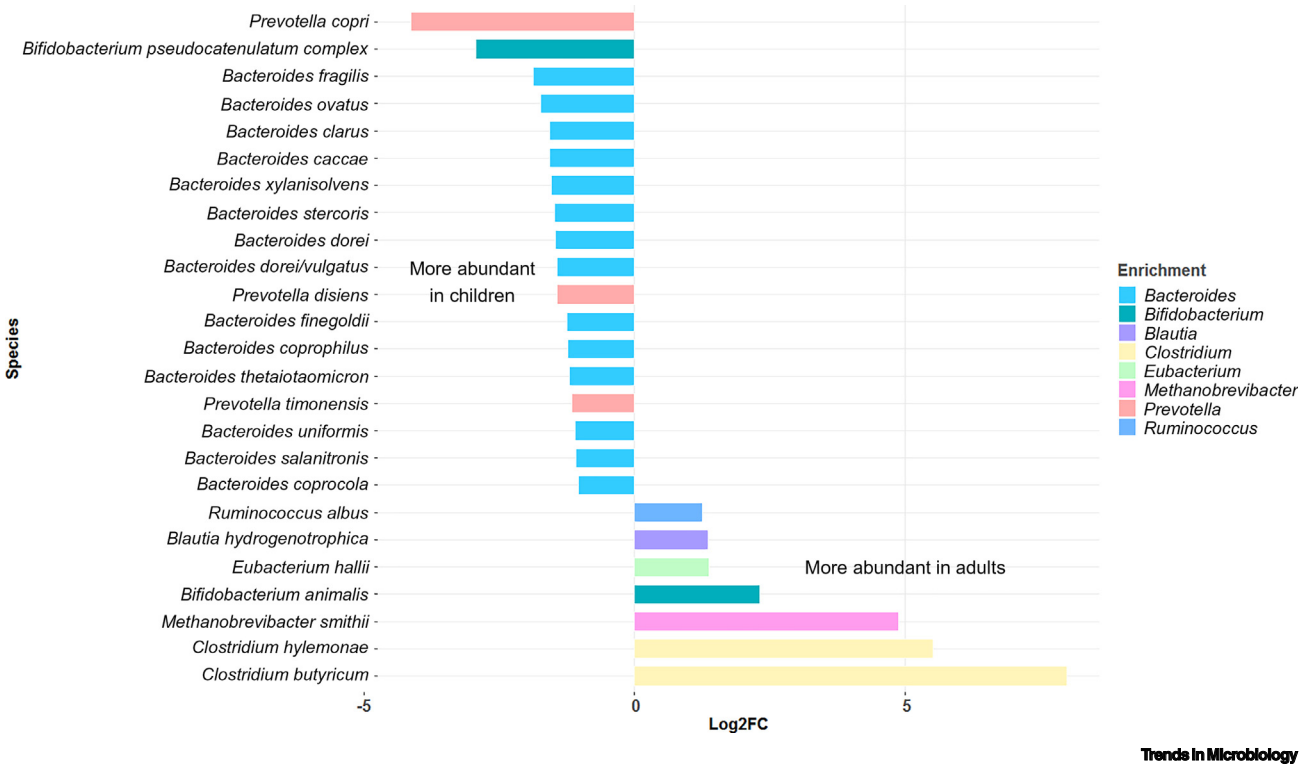

Figure 4. Varying Gut Microbiota in Children and Adults. Data retrieved from [46] based on metagenomic shotgun sequencing, at species level, on 281 Dutch school-age children (aged 6–9 years). The graph depicts selected major genera differing in abundance between children and adults (log_2-fold change >1, < −1).

Other inhabitants of the gut, including archaea, fungi, protozoa, and viruses, have received much less attention. Methanobacteriales is the most abundant archaeal order, and its members can produce methane, reducing CO_2 or methanol, with H_2 as the primary electron donor. The principal methanogens present in the gut microbiota are *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* [52]. A few studies have detected methanogens in pre-school or school-age children by order- or species-specific quantitative PCR. The prevalence of Methanobacteriales was found to be 65% in the children of one study (8–14 years old), a value lower than that reported in adults (89%) [53]. By contrast, another study reported that 88% of children (0–10 years of age) harbored *M. smithii*, and 11% harbored *M. stadtmanae* [54]. The largest exploratory study to date, on 472 children (aged 6–10 years) from the KOALA Birth Cohort Study, reported that 78.2% of children were colonized with *M. smithii*, and 8.3% with *M. stadtmanae* [55]. Shotgun metagenomics confirmed the lower prevalence of Methanobacteriales in children than in adults, with the detection of *M. smithii* in 98% of adults and 85% of children, and of *M. stadtmanae* in 43% of adults and 19% of children [46].

Fungal diversity has been largely neglected in most studies to date. Although much less abundant than bacteria (from 10^5 to 10^6 fungal cells per gram of fecal matter), fungal cells can be up to 100 times larger than bacteria, thereby making a significant contribution to the mass and metabolism of the microbiota [56]. One study analyzed changes in the abundance and prevalence of fungi with age and reported that infants and children under the age of 10 years had a richer fungal microbiota than adults, with *Aspergillus* and Tremellomycetes more prevalent in children than in adults [57].

Other components of the gut microbiota, such as phages, have been little studied, despite their clinical relevance [58,59].

In summary, although only a limited number of studies have been performed, with considerable differences in cohort size and follow-up duration, the data obtained to date suggest that the gut microbiota of pre-school and school-age children is similar to that of adults in terms of their global composition, but that some features are not yet fully developed (Figure 3). These differences are consistent with the notion that the gut microbiota continues to develop after the age of 3 years. The microbiota may develop more slowly in some children than in others, who may present an intermediate microbiota state. *Bifidobacterium* has consistently been reported to be more abundant in pre-school and primary school-age children, and even in older children [60], than in adults. These findings suggest that *Bifidobacterium* levels may gradually decrease until adulthood.

Human Gut Microbiota from Children across Populations

Major efforts are being made to catalog the variation in gut microbiota within and between populations. These studies have generated substantial insight into the environmental factors shaping the structure and function of the gut microbiota. The first large population-based study of the gut microbiota included 531 healthy individuals including infants (under the age of 3 years), children (aged 3–17 years), and adults, from several countries (Venezuela, Malawi, and USA), based on 16S rRNA gene sequencing [39]. This study highlighted significant maturation of the gut microbiome during the first 3 years of life, with high levels of inter-subject variation in all populations. Alpha-diversity [expressed as the number of operational taxonomic units (OTUs) present] was lower in children from the USA compared with that of non-American subjects, but only in subjects over the age of 3 years. This difference can be explained by the more rural lifestyle, divergent environmental exposure, or diet in Malawi and Amazonia compared with that in the USA. A high species richness of the gut microbiota, with specialization towards recalcitrant fiber

metabolism, was confirmed in children aged 1–6 years from Burkina Faso than in their age-matched Italian counterparts [61]. A few studies in children over the age of 3 years have compared the effect of westernization on the gut microbiota. The diversity, structure, and temporal stability of the fecal microbiota were compared between healthy children living in an urban slum in Bangladesh and age-matched American children [62]. The gut microbiota differed between Bangladeshi and American children, with greater overall diversity and enrichment in *Prevotella*, *Butyrivibrio*, and *Oscillospira,* reported for the Bangladeshi children [62]. The Asian Microbiome Project has made great efforts to explore the diversity of the gut microbiota in school-age children from different countries in Asia and from different cities within the same region. This project has resulted in the generation of gut microbiota profiles for 303 school-age children living in urban or rural regions in five Asian countries (China, Japan, Taiwan, Indonesia, and Thailand) [51]. Subsequent studies within this project addressed the effect of urbanization at particular sites within a given country (Thailand and the Philippines) [63,64]. Both studies converged towards findings similar to those reported for adults, with gut microbiota displaying higher richness and a greater prevalence of the *Prevotella* enterotype at rural sites than in cities, where people switch to a modern lifestyle [63,64]. Thus, differences in lifestyle, and westernization in particular, strongly influence the composition of gut microbial populations in children, as already reported for adults.

Filling Nutritional Gaps to Sustain Gut Microbiota Function

Dietary habits vary considerably around the world. Each country has its own lifestyle, culture, and eating habits, which are intrinsically associated with socioeconomic conditions. There are major differences between Asian, African, American, and European food. The 'Eastern' or 'Asian' diet is mostly consumed by the Chinese population and its Asian neighbors [65]. This diet is varied, consisting largely of raw fish, fried foods, rice or noodles, soup, and plant products, such as tofu, algae, bamboo shoots, or lotus roots. Fermented foods play a key role in the Asian diet, in the form of miso, kimchi, and natto, for example. The Western diet is consumed mostly by American and European populations, and an increasing number of populations that are undergoing modernization at the expense of their traditions. It consists of a combination of plant-based products and animal products, such as meat, fish, milk, and eggs. There has been a recent overall worldwide trend towards a diet relatively high in fat and processed foods and low in fiber, fresh fruits and vegetables, and carbohydrates. This diet is thus rather high in fat and sugar and low in plant polysaccharides [66–68]. The World Health Organization has recognized a real fiber deficit, known as the 'fiber gap', in many Westernized countries, in both adults and children [69,70]. Several organizations have recommended that adult women should eat 25 g of fiber per day, and adult men 30 to 40 g per day, almost double the current intake. In addition to increasing the amount of fiber eaten to fill this fiber gap, it is also important to increase the diversity of fiber sources. Too few relevant studies have been performed in infants and children to determine the appropriate daily intake of fiber and, thus, the fiber gap. Recommendations are often either based on extrapolations of adult data [69], or a formula consisting of the age plus 5 g fiber per day, or half the recommended intake of fiber for adults [71]. In general, the recommended dietary fiber intake for children ranges from 10 to 40 g per day, depending on age, sex, energy intake, and country [69].

The low fiber consumption associated with Westernization is associated with a decline in bacterial diversity in adults, with specific decreases observed in the abundance of certain taxa, such as *Prevotella*, *Oxalobacter*, Succinivibrionaceae, Paraprevotellaceae, and Spirochaetaceae [72–74]. In industrialized countries, the consumption of large amounts of fiber from diverse sources is correlated with a greater diversity [21–23] or stability of the gut microbiota in adults [75,76]. Increasing fiber content and diversity has been proposed as an approach to promoting

microbiota diversity and symbiosis with the host [77], as low levels of bacterial diversity are associated with various diseases [78–83].

One study revealed an association between the long-term dietary habits of healthy children aged 4–8 years and the composition and stability of the gut microbiota over a period of 6 months [84]. It also indicated that a dietary profile enriched in fiber-rich foods, such as vegetables, fruits, and grains, was associated with a higher degree of microbiota stability. In the KOALA Birth Cohort Study, the consumption of plant-based protein and dietary fiber in school-age children was found to be associated with microbiota variation but was driven by the *Prevotella* enterotype for plant-based protein and by the *Bacteroides* and *Prevotella* enterotypes for dietary fiber [47]. Studies of school-age children performed by the Asian Microbiome Project have reported that dietary fat consumption, which is higher in cities where there is a transition to a modern lifestyle and a switch to a Western diet (close to 30% fat, the maximum recommended by the World Health Organization), is correlated with *Bacteroides* and inversely correlated with *Prevotella* and *Succinivibrio* relative abundance [64]. However, overall, the relationship between dietary profile and microbiota composition has been little studied in children.

Plasticity of the Gut Microbiota in Children, and the Response to Dietary Intervention

It is generally agreed that the gut microbiota is relatively stable in the long term in adults [17,18,85]. Several studies in children have assessed microbiota stability without challenges (Box 1) or in response to external changes.

In particular, the gut microbiota has been followed in children exposed to two classes of antibiotic (macrolides and penicillins) [88]. The gut microbiota of children exposed to macrolides underwent a long-lasting (up to 2 years) shift, with a decrease in *Bifidobacterium* and an increase in Enterobacteriaceae and Bacteroidetes, accompanied by a decrease in species richness. Microbiota disruption was further associated with an increase in the risk of asthma and a predisposition to antibiotic-associated weight gain. The shift in the microbiota observed after treatment with penicillin was minimized by the daily intake of milk containing a probiotic strain for 7 months, whereas that induced by macrolide treatment was not [89]. The prevention of antibiotic-induced changes in the gut microbiota was observed following 6 months of inulin intake in children 3–6 years of age, especially for *Bifidobacterium* [90]. The consumption of a strain of *Lactobacillus rhamnosus* by children not exposed to antibiotics induced some changes in the microbiota (Table 1), contrasting with earlier findings in adults, indicating that its consumption led to only minor changes in the gut microbiota in healthy subjects [91]. Daily consumption of almonds in adults (42 g) and children (14 g) induced greater changes in the gut microbiota in children than in adults [92]. Another study monitored the response of the gut microbiota in seven urban subjects, including two children aged 3 and 7 years, following 16 days of immersion in an Amerindian village in the rainforest [93]. During their stay, they followed the local diet (low-fat/high-fiber unprocessed

Box 1. Stability of the Gut Microbiota of Healthy Pre-school and School-age Children

A few studies have monitored the evolution of the microbiota, without challenge, in the short and long term in young children. One study explored the temporal evolution of the gut microbiota of the same 61 Dutch children (2–18 years old) in the short term (weekly sampling for 6 weeks) and longer term (over a period of 18 months) [86]. The study reported an association between higher alpha-diversity and higher stability in children between the ages of 2 and 18 years. The stability of the gut microbiota varied with time and by phylum, with Bacteroidetes the most stable phylum, followed by Proteobacteria. Overall, compositional stability, defined as a cosine distance of 70%, was recorded over the 18-month monitoring period. This value is within the range of stability for adults reported in strain-tracking studies over 5 years [87]. This study provides additional insight into the dynamics of the gut microbiota in healthy pre-school and school-age children, but larger studies with a higher resolution are warranted to monitor the short- and long-term evolution of the microbiota in children as they grow up.

diet) and lifestyle. Interestingly, although the gut microbiota was altered in all subjects, its response to environmental change was more pronounced in children, in whom an increase in alpha-diversity was observed, whereas no such increase was observed in adults. This study, together with the earlier one [89], provided additional evidence to suggest that the microbiota of children may be more malleable to modification through changes in environment, including diet, than that of adults. In recent years, several interventional clinical trials have been performed in healthy children followed by analyses of the fecal microbiota (Table 1). The interventions mostly consisted of the ingestion of fiber and probiotics. Overall, the gut microbiota response converged towards the stimulation of endogenous *Bifidobacterium,* microbiota richness, or gut microbiota

Table 1. Overview of Clinical Studies Monitoring Gut Microbiota Responses to Dietary Interventions in Healthy Children[a]

Cohort description (age and number of children enrolled)	Type of study (design/ country of investigation)	Intervention (test product, daily dose, duration)	Observed results in test groups	Refs
Fiber/ prebiotics /other ingredients				
3–6 years *N* = 219	RDBPC Hungary	Inulin-type fructans 6 g/day 24 weeks	**Quantitative PCR** Increase in *Bifidobacterium* and *Lactobacillus*	[101]
3–6 years *N* = 209			**16S rRNA gene sequencing** Antibiotic treatment: stabilization of *Bifidobacterium*	[90]
4 years *N* = 29	RDBPC-CO USA	Almonds 14 g/day 3 weeks	**16S rRNA gene sequencing** Increase in *Bacteroides*, Lachnospiraceae, Ruminococcaceae	[92]
8–12 years *N* = 29	RDBPC-CO Belgium	Wheat bran 0 or 5 g/day 3 weeks	**Fluorescence in situ hybridization** Increase in *Bifidobacterium* (5 g/day)	[71]
10–13 years *n* = 19–21	RDB-CO USA	Galacto-oligosaccharides 0, 5 or 10 g/day 3 weeks	**Quantitative PCR** Increase in *Bifidobacterium* (5 g/day)	[102]
11–14 years *N* = 28	RDB-CO USA	Soluble corn fiber 0, 10, and 20 g/day 4 weeks	**16S rRNA gene sequencing** Increase in microbial diversity Increase in *Parabacteroides*	[103]
Probiotics				
2–7 years *N* = 88	RDBPC Finland	*Lactobacillus rhamnosus* GG in milk 4 x 10^8 CFU/day 7 months	**Phylogenetic microarray** No antibiotic treatment: increase in *Prevotella, Lactococcus,* and *Ruminococcus* Antibiotic treatment: decrease in *Escherichia* Smaller change in the microbiota with penicillin use	[89]
5.7 ±2.6 years *N* = 10	Open Italy	*Bifidobacterium breve* B632 and *Bifidobacterium breve* BR03 in oily suspension 10^9 CFU/day 21 days	**Cultivation** Increase in *Bifidobacterium*	[104]
7.7 ±2.4 years *N* = 23	Open Japan	*Lactobacillus casei* strain Shirota in milk 4 x 10^{10} CFU/day 6 months	**Quantitative PCR** Increase in *Bifidobacterium*	[105]

[a]RDBPC, randomized, double-blind, placebo-controlled clinical trial; RDB-CO, randomized, double-blind, crossover clinical trial; RDBPC-CO, randomized, double-blind, placebo-controlled, crossover clinical trial.

stabilization. It would be tempting to target ecological markers resembling those in adults (higher richness and diversity, stimulation of beneficial microbes), but it remains unclear which microbiota response should be favored in children: delaying or accelerating the transition to an adult microbiota.

Current efforts to understand the changes in the microbiota of children with intestinal symptoms, metabolic disease, undernutrition, and the response of these changes to dietary interventions [94–98] are not discussed here, but the findings of these studies will be highly relevant to determine the extent of deviations relative to healthy controls.

Concluding Remarks

We have witnessed an unprecedented increase in knowledge about microbiota variation and host association across life stages, geographies, and lifestyles. Several studies have highlighted the impact of various perinatal factors on gut microbiota development, with, in some cases, a time lag of up to few years after birth. Diet is a strong driver of microbiota maturation during the first year of life, with adaptation based on substrate availability. The microbiota undergoes most of its development very early in life, but recent studies have suggested that it continues to evolve after the age of 3 years. This suggests that childhood may provide additional opportunities for microbiome-based interventions to promote health or prevent microbiota deviation, notably through diet. Follow-up studies of birth cohorts should provide large amounts of information about this period of life. The inclusion of approaches with a higher resolution, focusing on the subspecies-strain level, and the integration of quantitative profiling, will make it possible to perform a detailed dissection of the effects of environmental factors on the microbiota. The enlargement of population-based cohorts across the world, coupled with phenotyping, should shed light on the factors associated with specific microbiota configuration and facilitate the development of microbiota-based dietary recommendations for children (see Outstanding Questions).

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Outstanding Questions

How stable are the strains of the gut microbiota of children in the short and long term?

How large and persistent are changes in the gut microbiota in response to environmental changes relative to those in adults, across populations?

What is the relationship between the composition of the gut microbiota in children and that in adults and the elderly?

What host or environmental factors govern the maturity of the gut microbiota in children?

Is it better to accelerate or delay the transition of the gut microbiota to a more adult-like composition?

Are delays in gut microbiota maturation associated with longer-term symptoms or disease in adults?

How do other members of the microbiota (fungi, phages) develop, and how do they influence bacterial development?

How feasible are microbiota-based interventions in children?

Is the gut microbiota from children more or less resilient to stressors?

How much microbiome modulation would be expected, or required, to affect the health of the host in later life?

What role will specific gut commensals and 'synthetic microbiomes' isolated from adults play as next-generation probiotics and therapeutic microbes in children?

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